KEFIR VINEGAR: ASSESSMENT OF METABOLIC ACTIVITY OF KEFIR BY BIOSPECKLE LASER

K.T. Magalhães-Guedes¹, R.O. Viana², R.A. Braga Jr.³, D.R. Dias⁴, I.L. Nunes⁵, R.F. Schwan⁶

1- School Nutrition, Federal University of the Bahia (UFBA), 40.110-150, Salvador-BA, Brazil, Phone: +55 (71) 3283-7700 – e-mail: (karynamagat@gmail.com)
2- Biology Department, Federal University of Lavras (UFLA), 37.200-000, Lavras-MG, Brazil, Phone: +55 (35) 3829-1613 – e-mail: (roberta.oliveiraviana@gmail.com)
3- Engineering Department, Federal University of Lavras (UFLA), 37.200-000, Lavras-MG, Brazil, Phone: +55 (35) 3829-1672 – e-mail: (robraga@prpg.ufla.br)
4- Food Science Department, Federal University of Lavras (UFLA), 37.200-000, Lavras-MG, Brazil, Phone: +55 (35) 3829-5256 – e-mail: (diasdr@dca.ufla.br)
5- School Nutrition, Federal University of the Bahia (UFBA), 40.110-150, Salvador-BA, Brazil, Phone: +55 (71) 3283-7700 – e-mail: (itaciara@yahoo.com)
6- Biology Department, Federal University of Lavras (UFLA), 37.200-000, Lavras-MG, Brazil, Phone: +55 (35) 3829-1613 – e-mail: (rschwan@dbi.ufla.br)

RESUMO – O objetivo deste estudo foi desenvolver um vinagre de maçã à base de kefir e estudar este processo de fermentação usando nova metodologia com Biospeckle Laser. Os grãos de kefir Brasileiros foram inoculados no mosto de maçã para a produção de vinagre. Os grãos de kefir foram analisados diariamente usando Biospeckle Laser e o valor da atividade biológica obtida foi de 14,21. A atividade biológica dos grãos de kefir diminuiu nos primeiros dias de processo de fermentação. A diminuição da atividade biológica persistiu até ao terceiro dia de fermentação. No quarto dia, a atividade biológica dos grãos de kefir no mosto de maçã aumentou para 11,51. No último dia de fermentação a atividade biológica dos grãos de kefir reduziu ao valor de 7,12. Os resultados mostraram que os grãos de kefir foram capazes de utilizar o mosto de maçã como substrato e produzir etanol e ácido acético. O rendimento de ácido acético no vinagre de kefir foi ~ 79%. A concentração de ácido acético foi ~ 41.00 gL⁻¹, atingindo o padrão exigido pela legislação Brasileira aceita como vinagre (ácido acético 4,0%). Vinagre de kefir mostrou boa aceitação em análise sensorial. Este é o primeiro estudo a produzir vinagre de kefir. A metodologia para medir a atividade metabólica de grãos de kefir por Biospeckle Laser facilita o controle microbiológico ao longo de um processo de fermentação.

ABSTRACT – The aim of this study was to develop a kefir apple-based vinegar and study this fermentation process using new methodology with Biospeckle Laser. Brazilian kefir grains were inoculated in apple must for vinegar production. Kefir grains were analyzed daily using Biospeckle Laser and the biological activity value obtained was 14.21. The biological activity of the kefir grains decreased in the early days of the fermentation process. The decrease in biological activity persisted until the third day of fermentation. On the fourth day the biological activity of kefir grains in apple must increased to 11.51. On the last day of fermentation the biological activity of kefir grains decreased to value 7.12. The results showed that kefir grains were able to utilize apple must as substrate and produce ethanol, and acetic acid. The yield of acetic acid in the kefir vinegars was ~79%. The acetic acid concentration was ~41.00 gL⁻¹, reaching the required standard for the Brazilian legislation accepts it as vinegar (4.0% acetic acid). Kefir vinegar showed good acceptance in the sensory analysis. This is the first study to produce kefir vinegar. The methodology to measure the
metabolic activity of kefir grains by Biospeckle Laser facilitates the microbiological control over a fermentation process.

PALAVRAS-CHAVE: análise química; ácido acético; maçã.

KEYWORDS: chemical analysis; acetic acid; apple.

1. INTRODUCTION

Kefir is a culture employed to produce beverages, such as the traditional Russian beverage also named "kefir" which is from milk, and has low alcohol content (Simova et al., 2002, Magalhães et al., 2010, 2011). The kefir is a mixed culture of various yeast species of the genus Kluyveromyces, Candida, Saccharomyces and lactic acid from bacteria of the genus Lactobacillus combined in a matrix of proteins and polysaccharide 'kefiran', which are formed during cell growth under aerobic conditions (Simova et al., 2002, Magalhães et al., 2010, 2011). The kefir beverage contains vitamins, minerals and essential amino acids that help the body with healing and maintenance functions and contains easily digestible complete proteins (Simova et al., 2002, Magalhães et al., 2010, 2011).

An optical technique with potential use in biological metrology, particularly in biological activity, is the dynamic Laser Speckle, or Biospeckle, when associated to biological material (Guedes et al., 2014). When a laser beam is scattered by a biological sample, the scattered waves generated in the illuminated sample create the speckle pattern that changes its image in accordance with the changes in the monitored material. Thus, the surface appears to be covered with tiny bright dots that fluctuate in a seemingly random way as for a boiling liquid.

The aim of this study was to develop an apple vinegar-based kefir and study this fermentation process using new method Biospeckle Laser.

2. MATERIALS AND METHODS

Brazilian kefir grains (Stock-culture of the Microbiology laboratory of the Federal University of Lavras, Brazil) were used in the experiments. The apple fruits were obtained from Lavras city market, Minas Gerais State in of Brazil. These apple fruits, which fail to meet the quality standards required for marketing, were washed in clean water to remove residues. The pulp was extracted using an automatic depulping machine (ITAMETAL 0.5 DS, Itabuna, BA, Brazil). The ° Brix was analysed, and the juice was divided into three 500 mL Erlenmeyer flasks to start batch fermentation.

2.1 The process of vinegar production

The initial apple must was inoculated with kefir grains in a proportion of 10% w/v. The flasks were incubated statically at a temperature of 28 °C and fermentation was monitored daily to observe the end of the fermentation (stability of the consumption of sugars - Brix). The fermentation must was filtered by kitassato filters (0.5 µm). The kefir grains were recovered. The alcoholic must was used for apple kefir vinegar production. To determine fermentation performance were calculated the substrate conversion factors in ethanol (Yp/s), substrate conversion in glycerol (Yg/s), substrate conversion in acetic acid (Yac/s), ethanol volumetric productivity of ethanol (Qp), biomass productivity (Px) and conversion efficiency (Ef). The total concentration of sugars was calculated considering the conversion for each mole of sucrose (342 g) in 1 mol of glucose (180 g) and 1 mol of fructose (180).

The apple fermented must obtained in the previous section were acetified in 500 mL Erlenmeyer flasks, which were controlled temperature of 28 °C and agitation of 150 rpm. The acetic
fermentation was conducted using the following treatments: 1. kefir grains 10% (w/v); 2. kefir grains 20% (w/v). During acetic fermentation, daily samples were taken (6 days) in triplicate for acidity analysis (pH meter) and alcohol (alcoholometer). The acetic fermentation was finish on the sixth day when the vinegar presented alcohol content below 1.0% (v/v). The yield was calculated as the acetic acid produced in relation to the theoretical yield. The theoretical yield was calculated as the amount of ethanol converted to acetic acid, in which 1.0 g of ethanol yields 1.304 g of acetic acid. The kefir apple vinegar was then filtered through diatomaceous earth, bottled and pasteurised at 65 °C for 20 min. Samples were analysed by optical microscopy (National Optical 131 Microscope) to evaluate the clarity of the vinegar.

2.2 Assessment of metabolic activity by Biospeckle Laser

Daily analysis of kefir grains were done by Biospeckle Laser to observe the biological activity. The grains of kefir were illuminated by a HeNe laser, wavelength of 632 nm, and 10 mW power, enlarged by a plane concave lens in order to cover the entire sample. The interference patterns formed on them were captured by a CCD camera 640 × 486 pixels, with a shutter speed of 1/60 s and in a rate of 0.08 s creating a collection of 128 images. The analysis of the images, from the laser illumination, was performed according Guedes et al. (2014).

2.3 Sensory evaluation

The final vinegar were evaluated in a sensory test. The tasters were asked to indicate how much they liked or disliked each product on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) according to overall acceptability (Moraes, 1993) by twenty-five untrained tasters, both males and females, 25–35 years of age (students and staff of the Biology Department, Federal University of Lavras, Brazil). The evaluation of the appearance, color and odor attributes were also conducted. Randomised, refrigerated (10 °C) 10 mL samples were served in clear, tulip-shaped glasses with a volume of 50 mL. These samples were marked with a three digit random number and covered with Petri dishes.

2.4 Statistical analyses

Each fermentation process (alcoholic and acetic) was conducted in triplicate and the mean values ± standard deviations are reported. The Tukey's test was performed using Statgraphics Plus for Windows 4.1 software (Statistical Graphics Corp. Software - (Free download)) to evaluate the statistical significance (level of P < 0.05).

3. RESULTS

Kefir grains were analyzed daily using Biospeckle Laser and the biological activity value obtained was 14.21. This value was considered the initial fermentation time (0h). The test of homogeneity of the grains of kefir can be seen in Figure 1, where the in Figure 1a the image of the kefir grain is presented with an illustration of a window where the laser was illuminated and the images assembled.

The result of the biological activity test is presented in Figure 1b,c. In the alcoholic fermentation the biological activity of the kefir grains decreased in the early days of the fermentation process (Figure 1b). The decrease in biological activity persisted until the third day of fermentation. On the fourth day the biological activity of kefir grains in apple must increased to 11.51. On the last day of fermentation the biological activity of kefir grains decreased to value 7.12.

The acetic fermentation occurred simultaneously as the alcoholic fermentation. In the acetic fermentation the biological activity of the kefir grains decrease in the early days of the fermentation
process for treatment 1 and 2 (Figure 1c). The decrease in biological activity persisted until the third day of fermentation. On the fifth day, the biological activity of kefir grains increased to 8.01 (treatment 1) and 8.79 (treatment 2). On the last day of fermentation the biological activity of kefir grains decreased to 6.36 (treatment 1) and 6.77 (treatment 2).

Figure 1 - Biological activity of kefir grains by Biospeckle Laser technique. (A) Kefir grains and image formed by Biospeckle Laser. (B) Alcoholic fermentation ( ). (C) Acetic fermentation, ( ) 10% kefir inoculum, ( ) 20% kefir inoculum.

The apple alcoholic must was produced. The fermentation temperature selected was 28 °C and the kefir grains were chosen as the inoculum for the fermentation process. An inoculum of 10% kefir inoculum was sufficient because the ethanol concentration at 120 h of fermentation was approximately 97.0 g L$^{-1}$ (~12.3 °GL (Gay Lussac)). The pH value was 3.82 and “Brix was 4.37 in the final alcoholic must. The values of the kinetic parameters obtained from the apple alcoholic must are described as ethanol productivity ($Y_p$) $0.47$ g, yield glycerol ($Y_g$) $0.05$ g g$^{-1}$, ethanol yield ($q_p$) $0.37$ g L$^{-1}$ h and fermentation efficiency (Ef) 93.12%.

After the alcoholic fermentative process, the alcoholic must was processed as described in the methodology for the subsequent production of apple vinegar. The yield of acetic acid in the kefir vinegars was ~79%. The total acetic acid concentration was ~41.00 gL$^{-1}$, reaching the required standard for the Brazilian legislation accepts it as vinegar (4.0% acetic acid) (Brasil, 2009). The kefir vinegars showed the clear appearance and had a good colour (pale yellow).

Sensory analyses were conducted to kefir vinegar (10% and 20% of kefir grain inoculum) and the commercial apple vinegar. Using the CATA testing, the vinegars were subjected to sensory evaluation to assess their acceptance and preference. Vinegars at 10%, 20% and commercial had good acceptance (scores 7.1, 7.4 and 7.2, respectively). Consumers classified vinegars in "liked slightly". Vinegars were evaluated as "sour, acetic acid, limpid and translucent appearance" (Figure 2). No significant differences observed between all evaluated vinegars. The apple kefir vinegars produced were well accepted by the evaluators (95% of consumers would buy kefir vinegar).
4. DISCUSSION

The apple pulp was processed to obtain a fermentable must from which the alcoholic must was produced. The inoculum for the fermentation process (kefir grains) was sufficient because the ethanol concentration at 120 h of fermentation was approximately 97.0 g L\(^{-1}\) (~12.3 °GL (Gay Lussac)). These results are comparable to that obtained by Dias et al. (2007) who found a value of 89.5 g L\(^{-1}\) when fermenting cocoa pulp with an initial 22 °Brix. Acetic acid was formed during the fermentation of apple alcoholic must. Acetic acid provides a pleasant taste and inhibits the development of undesirable or pathogenic microorganisms, due to the high acidity.

Kefir grains were also analysed by the Biospeckle Laser technique. The results presented the activity of the kefir grains during fermentation (Figure 1), where it was possible to observe the sensitivity of the technique to follow the expected activity. The proposed technique is simple, relatively cheap and fast, easy to implement, and requires only a laser and standard digital imaging processing hardware components (Guedes et al., 2014). The application of Biospeckle Laser method to the kefir viability detection was one of objectives of this work, but the optical technique could also be applied to characterise the kefir grains in other types of processes. Biospeckle Laser technique showed an efficient tool for monitoring and quantifying biological activity of kefir grains, showing the viability time of these grains in a fermentation process, which demonstrates the technique potential for evaluating and monitoring of kefir grains in production of kefir vinegars in industrial scale.

In our study, we studied a new technology for vinegar production, which was conducted using kefir grains in a submerged culture fermentation process using kefir grains at 10% inoculum (treatment 1) and kefir grains at 20% inoculum (treatment 2). The acetic acid concentration was ~41.0 g L\(^{-1}\) in both treatments. Therefore, the kefir grains culture was found to have efficiently fermented the apple alcoholic must to produce vinegar. The taste of the filtered vinegar was fruity and an nice smell was noted. Various organic acids in vinegar are important for imparting a suitable taste and flavour. The total acetic acid concentration in the apple vinegar produced using kefir grains inoculum was ~41.0 g L\(^{-1}\), which is approximately two times the concentration in onion vinegar (~25.0 g L\(^{-1}\)) produced using cell-free of *Acetobacter aceti* (Simova et al., 2002). The kefir grains were highly satisfactory for acetic fermentation using apple alcoholic must as the medium. This system achieved higher acetic acid productivity than that of fermentations by free cells. Our results indicated that the
use of kefir grains might be one of the best fermenting strategies employed to overcome substrate limitation and achieve high product yield.

The period for the oxidation of ethanol to yield acetic acid was 140 h. According to the stoichiometry of reaction that converts ethanol to acetic acid, 1.0 g of ethanol can provide 1.304 g of acetic acid. In industries, the conversion of 1.0 g ethanol to 1.0 g of acetic acid (yield of 76.6%) is considered economic (Dias et al., 2007). The production was favourable, reaching values of ~79.0% yield. Based on the analyses of the vinegar and the limits required by law (Brasil, 2009), the final product of kefir vinegar has an acceptable acetic acid level, of approximately 5.0% (w/v), and an ethanol concentration of less than 1.0% (v/v). The kefir vinegar proved to be a very promising product.

5. CONCLUSION

This is the first study to produce kefir vinegar. A new methodology to measure the metabolic activity of kefir grains by Biospeckle Laser was presented. This fact facilitates the microbiological control over a fermentation process. Kefir vinegar was well accepted by sensory analysis. The technology proposed in this study is significant. The key point for industrial application of the proposed technology is the promotion of fermentation by an immobilised-cell biomass (kefir grains) that provides the possibility of eliminating the use of centrifugal separators, which have a high energy demand and require high industrial investment.

6. ACKNOWLEDGEMENTS

The authors thank the CAPES, CNPq, and FAPEMIG for financial support and scholarships.

7. REFERENCES


